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Pomegranate and Blueberry Extracts Ameliorate Gentamicin-induced Nephrotoxicity in Rats

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Citation

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ABSTRACT

Gentamicin (GM), an aminoglycoside antibiotic, is routinely prescribed in most bacterial infections. It generates free radicals that are mediators of nephrotoxicity. This work designed to assess the potential protective action of pomegranate (PG), blueberry (BB), and their mixture (PG + BB) extracts against GM-induced nephrotoxicity in rats. Rats (n=30) were classified into 5 groups (n = 6) 1- Control, 2- GM; rats intraperitoneal (i.p.) injected with GM (100 mg/kg) for ten days. Groups 3-5; rats were ingested (500 mg/kg b.wt) PG, BB, or PG + BB, respectively for 28 d before GM injection, and 10 days thereafter. Kidney functions, protein metabolism parameters, and ionic electrolyte (sodium and potassium) levels were estimation in serum. GM injection induced nephrotoxicity marked by significant elevate in kidney functions, total protein, and albumin levels, with significant decline in ionic electrolyte levels compared with the control group. Histopathological examination of renal tissues showed severe interstitial nephritis, cystic dilatation of renal tubules, thickening and dilatation of Bowman's space. Oral administration of PG, BB and PG+BB protected kidney from damage induced by GM as evidenced from histopathological and biochemical parameters improvements. The most effective treatment was PG+BB compared with the other pretreated groups. In conclusion, a combination of PG + BB has potent nephroprotective effects; therefore it may be useful for patients with kidney diseases or who receiving GM therapy.

Keywords: Pomegranate, blueberry, extract, gentamicin, nephrotoxicity, rats.

1. INTRODUCTION

Nephrotoxicity represents a major health problem, it takes place when kidney specific detoxification and excretion do not done appropriately, and a majority of kidney function is damaged (Kim and Moon, 2012). Aminoglycosides are potent bactericidal antibiotics that work as potent inhibitor of protein synthesis in various species of bacteria (Oliveira et al., 2009). Aminoglycosides are innate nephrotoxic drugs that may cause kidney injury with therapeutic doses (Perazella, 2018). Multiple studies indicated the role of reactive oxygen species in gentamicin-induced nephrotoxicity (Jensen et al., 2012; Cekmen et al., 2013; Perazella, 2018). Medicine-induced nephrotoxicity remains a main issue in the medical field, where usage of antibiotics is most of time unavoidable. Inappropriately use of these drugs leads to risk for potential side effects in renal (Jensen et al., 2012). The increases rate of renal diseases has raised the sense of concern among healthcare providers who believe that prevention is the best way to control the onset of the diseases and even to cure the disease (Asgary et al., 2014). In response to this, the medicinal potential of a lot of plants have been explored. There are many evidences indicate that free radicals are responsible renal toxicities and induce renal

vasoconstriction. A great body of literature support the effect of antioxidants in scavenging reactive oxygen metabolites and thus protecting from GM-induced renal failure (Cekmen et al., 2013).

Punica granatum L. fruit commonly called Pomegranate (PG), it belong to Punica L. genus, Punicaceae family (Holland et al., 2009). Blueberry (BB) (*Vaccinium spp.)* belongs to Ericaceae family, Vaccinium genus (Zhang et al., 2019). The PG and BB have highest antioxidant capacity among fruits. They contain the bioactive compounds polyphenols such as anthocyanin, leuco anthocyanins, tannins, alkaloids, catechins and flavonoids, which are used in several studies to assess their effectiveness for the cure and protect of many ailments (Zarei et al., 2011; Sadeghipour et al., 2014). Studies have demonstrated the anti-inflammatory, antioxidant, anticancer, and antimicrobial properties of PG and BB (Miguel et al., 2010; Nair et al., 2014; Mansouri et al., 2016). Despite few studies on the effect of PG or BB on nephrotoxicity, a comparison of their mixture effectiveness on the kidney has not been reported. Therefore, this study aimed to determine nephroprotective effects of PG and BB, and their mixture extracts against GM-induced nephrotoxicity in rats.

2. MATERIAL AND METHODS

Drugs, kits and chemicals

Gentamicin (1ml of gentam-80 contains gentamicin sulphate equivalent to 40 mg gentamicin base) was obtained from King Abdulaziz University Hospital Jeddah, KSA. All kits were purchased from Al Shafei Establishment (For Medical & Scientific Equipment), Jeddah, KSA. All chemicals were obtained from Sigma-Aldrich (St Louis, MO, USA).

Preparation of fruits

Fresh PG (*Punica granatum L.)* and imported BB (*Vaccinium sp*.) were obtained from local market in Jeddah, KSA. The PG pulp and BB sliced were froze (-30 °C for 4 h), dried by using freeze-dried machine (20 h), and grind (relative humidity 35%). The resulting dry powders were vacuum-sealed and stored at 4 °C (Seymour et al., 2011).

Fruits extraction

Each fruit powder (500 g) was mixed in a flask with 2 liters of ethanol 70%, stirred for 72 h, filtered by using funnel covered, then concentrated using rotary evaporator (Mansouri et al., 2016). The sticky residue was stored at 4 °C until used in experimental animals.

Animals

Male Wister albino rats (n=30, 110-140g) were purchased from the animal experimental unit of King Fahd Medical Research Center, KAU. They were allowed to one week for acclimatization in standard animal housing laboratory conditions. They fed standard diet and drinking water *ad libitum*. The experimental study was conducted from May 2019 to August 2019.

Induction of nephrotoxicity by GM

Nephrotoxicity induced by i.p. injection with GM (100 mg/kg) for 10 consecutive days (Amini et al., 2012).

Experimental design

Rats (n=30) were randomly allocated to five groups (6/each) including 1 (control); rats received water orally for 28 days, then they were injected with saline for 10 days. 2 (GM); rats received water orally for 28 days, after that they were injected with GM (100 mg/kg) for 10 consecutive days to induce nephrotoxicity. 3 (PG + GM); rats received daily PG extract (500 mg/kg). 4 (BB + GM); rats received daily BB extract (500 mg/kg). 5 (BB + PG + GM); rats received daily mixture of BB +PG (500 mg/kg). Groups 3-5 received PG, BB or their combination for 28 days then injected with GM for 10 consecutive days thereafter.

Biological evaluation

During the experiment animals initial weight (IBW), final body weight (FBW), and feed intake (FI) were recorded.

Body weight gain percent (BWG %) was calculated using this equation: $\mathrm{BWG\%} = \frac{\mathrm{FBW-IBW}}{\mathrm{IBW}} \text{ x } 100$

Feed efficiency ratio (FER) was calculated using this equation

$$\mathrm{FER} = \mathrm{Gain\ in\ BW\ (g)/Feed\ intake\ (g)}$$

After 24 h from the last dose of GM injection all rats after fasted overnight, the blood samples withdrawn from the retro orbital plexus, and centrifuged (3000 rpm for 15 min) to separate serum, which stored at -20° C until biochemical analysis. Then, the rats were sacrificed, and their kidney were weighted and carefully dissected out for histopathological examination, it fixed immediately in 10% formalin.

The relative kidney weight was calculated by: $\text{Relative kidney weight} = \frac{\text{Kidney weight}}{\text{Body weight}} \text{ x } 100$

Biochemical analysis

Serum samples were used for estimation of kidney function (creatinine, blood urea nitrogen and uric acid); protein metabolism parameters (total protein, and albumin, while globulin and albumin to globulin ratio (A/G ratio) were calculated); and ionic electrolyte (sodium (Na^+) and potassium (K^+)) using colorimetric assay kits as claimed by the manufacturer.

Histopathological examination

Kidney tissues were fixed in neutral buffered formalin (10%), stained with haematoxylin & eosin, and examined microscopically.

Statistics

Data were represented as a mean ± standard deviation (SD). The significance of the data was set at $p \leq 0.05$. The data were analyzed using a software system SPSS for Windows, Version 25.

3. RESULTS

Impact of PG, BB and their combination on biological evaluation in nephrotoxic rats

The GM group revealed significant decrease (p ≤ 0.001) in FBW, BWG%, FI and FER relative to the control group. Pre-treatment with BB exhibit a significant increase in the FBW, BWG% and FI (p ≤ 0.05, p ≤ 0.05, p ≤ 0.001, respectively) relative to the GM group. Pre-treatment with PG+BB exert significant improvement in FBW, BWG%, FI, and FER (p ≤ 0.001, p ≤ 0.001, p ≤ 0.05, and p ≤ 0.001, respectively) compared with the GM group. Significant elevation in the FBW, BWG%, FI, and FER was observed between the PG+BB+GM and the PG+GM groups, besides in FI and FER compared with the BB+GM group Table 1.

Table 1 Impact of PG, BB and their combination on biological evaluation in nephrotoxic rats

Groups	IBW (g)	FBW (g)	BWG %	FI (g/rat/day)	FER
Control	126.17±10.94	272.5±13.85	115.97±12.28	22.51 ± 1.53	0.172 ± 0.027
GM	125.0 ±10.96	196.17±10.57 $^{a\wedge}$	56.69±6.43 $^{a\wedge}$	19.18 ± 1.77 $^{a\wedge}$	0.098 ± 0.015 $^{a\wedge}$
PG + GM	123.33±8.17	181.83±10.82	47.43±11.45	18.48 ±1.80	0.083 ± 0.013
BB + GM	124.67±7.66	214.17±13.83 $^{b*, c\wedge}$	71.79±8.28 $^{b*, c\wedge}$	24.61 ± 2.33 $^{b\wedge\ c\wedge}$	0.096 ± 0.014
PG+BB + GM	123.0 ±8.60	222.0±17.31 $^{b\wedge, c\wedge}$	80.49±5.66 $^{b\wedge, c\wedge}$	20.81 ± 1.74 $^{b*c\#\ d\wedge}$	0.125 ± 0.011 $^{b\wedge,c\wedge,d\#}$

Values are presented as mean ± SD (n = 6). Significant difference from ^{a} control; ^{b} GM; ^{c} PG+GM; ^{d} BB+GM (*p ≤ 0.05, $^\#$ p ≤ 0.01 and $^\wedge$ p ≤ 0.001).

Impact of PG, BB and their combination on relative kidney weight in nephrotoxic rats

There were significant elevation in kidney weight and relative kidney weight in the GM group (p ≤ 0.001) relative to the control rats. There were significant decreases in the kidney weights and relative kidney weight in all nephrotoxic pretreated groups with PG, BB and their combination relative to the GM group. The BB+PG+GM group exert a significant decline in relative kidney weight relative to the PG+GM and BB+GM groups (p ≤ 0.01 and p ≤ 0.05, respectively) Table 2.

Table 2 Impact of PG, BB and their combination on kidney weight and relative kidney weight in nephrotoxic rats

Groups	Kidney weight (g)	Relative kidney weight
Control	0.762±0.097	0.279 ± 0.045
GM	1.151±0.155 $^{a\wedge}$	0.587 ± 0.089 $^{a\wedge}$
PG + GM	0.871±0.178 $^{b\#}$	0.479 ± 0.051 ^{b*}
BB + GM	0.928±0.176 ^{b*}	0.433±0.088 $^{b\#}$
PG+BB + GM	0.759±0.159 $^{b\wedge}$	0.342±0.065 $^{b\wedge, c\#, d*}$

Values are presented as mean ± SD (n = 6). Significant difference from ^{a} control; ^{b} GM; ^{c} PG+GM; ^{d} BB+GM (*p ≤ 0.05, $^\#$ p ≤ 0.01 and $^\wedge$ p ≤ 0.001).

Impact of PG, BB and their combination on serum kidney functions levels in nephrotoxic rats

It is clear that GM group had significant ($p \leq 0.001$) increases in the serum levels of creatinine, blood urea nitrogen, and uric acid concentrations (1.79 ±0.17, 44.83±5.34, and 7.95±0.35 umol/L, respectively) relative to the control group (0.60 ± 0.16, 17.10 ± 3.20, and 5.02 ± 0.96 umol/L, respectively). However, oral administration of PG and BB extracts and their combination decreased the elevated serum level of creatinine, blood urea nitrogen and uric acid. There were significant ($p \leq 0.001$) differences between all pretreated groups and the GM group. Besides, there was a significant ($p \leq 0.01$) reduce in serum creatinine and blood urea nitrogen levels in nephrotoxic rats received PG relative to the BB+GM group. There were significant decreases in creatinine, blood urea nitrogen, and uric acid levels in nephrotoxic rats received BB+PG when compared with BB+GM. The mixture of PG + BB was the most effective pretreatment compared with either PG or BB alone (Figure 1).

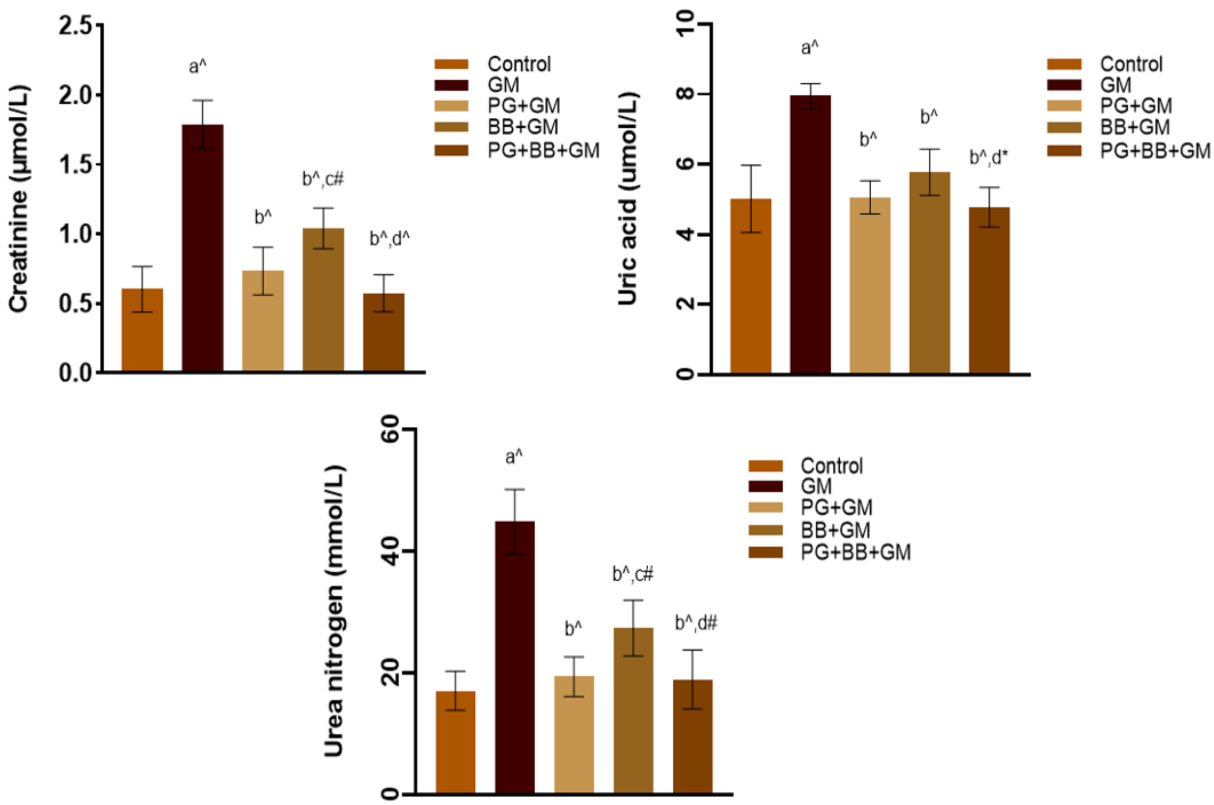

Figure 1 Impact of PG, BB and their combination on serum kidney function levels in nephrotoxic rats.
Values are presented as mean ± SD (n = 6). Significant difference from ^{a} control; ^{b} GM; ^{c} PG+GM; ^{d} BB+GM ($^{*}p \leq 0.05$, $^{\#}p \leq 0.01$ and $^{\wedge}p \leq 0.001$).

Table 3 Impact of PG, BB and their combination on serum levels of protein metabolism parameters in nephrotoxic rats

Experimental groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control	7.18 ± 0.7	3.82 ± 0.3	3.36 ± 0.164	1.16 ± 0.04
GM	9.29 ± 0.4 $^{a\wedge}$	5.89 ± 0.1 $^{a\wedge}$	3.40 ± 0.151	1.75 ± 0.09 $^{a\wedge}$
PG + GM	7.32 ± 0.8 $^{b\wedge}$	4.12 ± 0.5 $^{b\wedge}$	3.20 ± 0.14 ^{b*}	1.52 ± 0.14 $^{b\wedge}$
BB + GM	8.52 ± 0.4 $^{b*\ c\#}$	4.88 ± 0.7 $^{b\wedge\ c\#}$	3.64 ± 0.19 $^{b*\ c\#}$	1.42 ± 0.12 $^{b\wedge}$
PG+BB + GM	7.18 ± 0.5 $^{b\wedge\ d\#}$	4.44 ± 0.4 $^{b\wedge}$	2.74 ± 0.18 $^{b\wedge,\ c\wedge,d\wedge}$	1.82 ± 0.13 $^{c\wedge,d\wedge}$

Values are presented as mean ± SD (n = 6). Significant difference from ^{a} control; ^{b} GM; ^{c} PG+GM; ^{d} BB+GM ($^{*}p \leq 0.05$, $^{\#}p \leq 0.01$ and $^{\wedge}p \leq 0.001$).

Impact of PG, BB and their combination on serum levels of protein metabolism parameters in nephrotoxic rats

Serum total protein, albumin and A/G ratio levels revealed significant ($p \leq 0.001$) increases, however globulin level showed non-significant increase in the GM group relative to the control group. Pretreatments of nephrotoxic groups revealed significant improve in protein metabolism parameters. The improvement in total protein, albumin, and globulin showed significant differences

compared with the GM group for all pretreatments used. The BB+GM group revealed significant ($p \leq 0.01$) differences in in total protein, albumin, and globulin relative to the PG+GM group, as well as the PG+BB+GM group recorded significant differences in total protein, globulin, and A/G ratio relative to the BB+GM group Table 3.

Impact of PG, BB and their combination on serum levels of ionic sodium and potassium

In term of serum level of Na^+ and K^+, results of experimental groups are illustrated in Figure 2. Injection with GM exert significant ($p \leq 0.001$) decreases in the serum levels of Na^+ and K^+ relative to the control group. Oral administration of PG and BB extracts increase the serum level of Na^+ and K^+, there were significant differences ($p \leq 0.001$) between GM group and all pretreated groups PG+GM, BB+GM and BB+PG+GM (500 mg/kg b.wt.). An improvement response was observed with various pre-treatments, where a significant difference in Na^+ and K^+ level between the BB + GM group relative to the PG+GM group. The BB+PG+GM group revealed significant ($p \leq 0.001$) differences relative to the BB+GM group in serum levels of Na^+ and K^+.

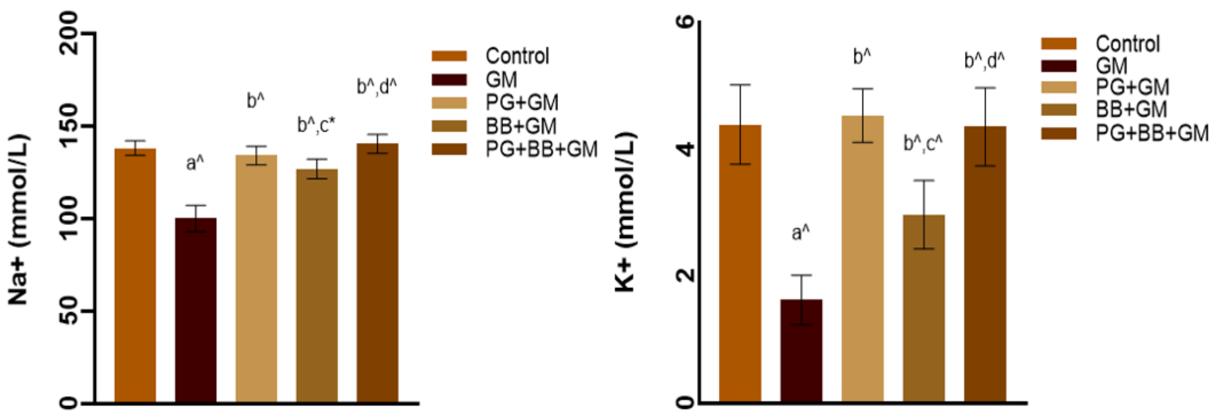

Figure 2 Impact of PG, BB and their combination on serum levels of ionic sodium (Na^+) and potassium (K^+) in nephrotoxic rats. Values are presented as mean ± SD (n = 6). Significant difference from [a] control; [b] GM; [c] PG+GM; [d] BB+GM ($^*p \leq 0.05$, $^\# p \leq 0.01$ and $^\wedge p \leq 0.001$).

Histopathological results

Microscopically, a photomicrograph of the kidney sections of rats from control group revealed normal histological structure of renal parenchyma, glomeruli and renal tubules, renal cortex, with narrow Bowman's capsular space in the glomerulous Figures (3.A and 4.A). Meanwhile, kidney sections of rats from the GM group showed severe histopathological alterations confined as marked interstitial nephritis with mononuclear inflammatory cells infiltration, cystic dilatation of renal tubules, hypercellularity of glomerular tuft, thickening of the parietal layer of Bowman's capsule, and dilatation of Bowman's space Figures (3.B and 3.C) and (4.B and 4.C). Examined kidney sections of rats from the GM group pretreated with PG showed few inflammatory cells infiltration, few congestion of renal blood vessels, glomerular tufts and slight dilatation of Bowman's space Figures (3.D and 4.D). Examined kidney sections of rats from the GM group pretreated with BB showed mild interstitial nephritis and few congestion of glomerular tufts Figures (3.E and 4.E). On the other hand, kidney sections from the GM group pretreated with BB+PG showed marked regression of the histopathological lesions Figures (3.F and 4.F).

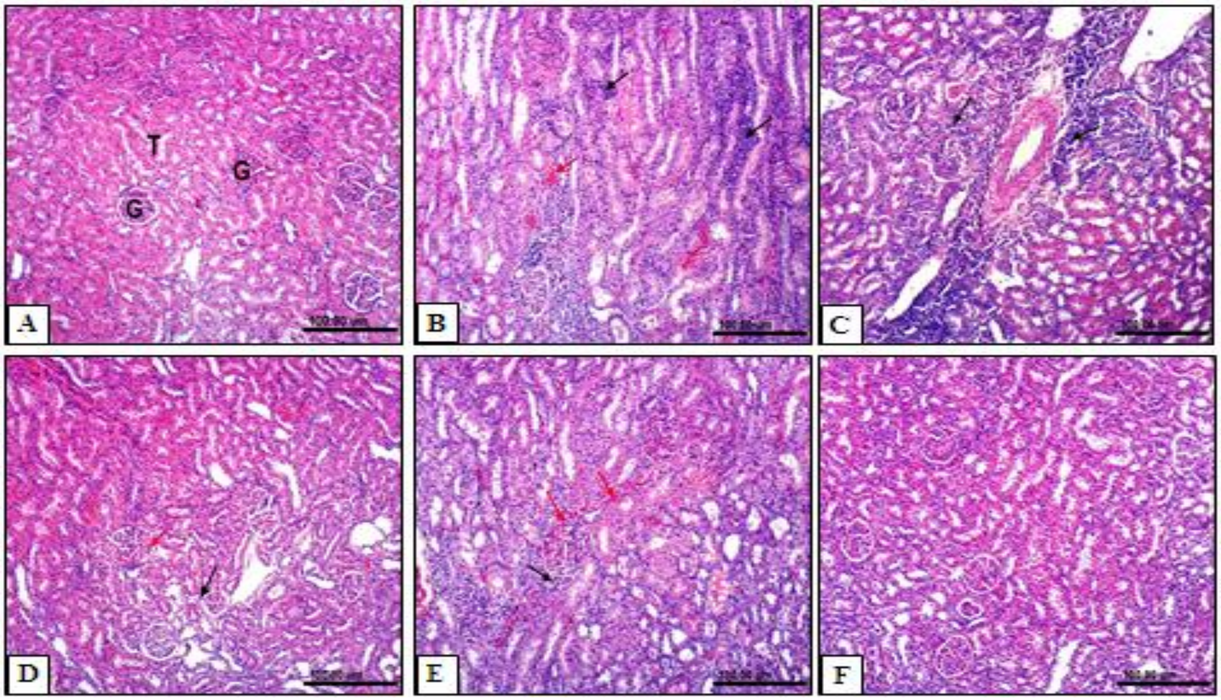

Figure 3 Impact of PG, BB and their combination on kidney histopathological alteration in nephrotoxic rats (H&E X100). A) Photomicrograph of a kidney section of rats from the control group showing normal histological structure of renal parenchyma, glomeruli (G) and renal tubules (T) (Fig. A). The GM group show marked interstitial nephritis (black arrow), and congestion of renal blood vessels (red arrow) (Fig. B-C). PG+GM group show few inflammatory cells infiltration (black arrow) and congestion of glomerular tuft (red arrow) (Fig. D). The BB+GM group showing few inflammatory cells infiltration (black arrow) as well as congestion of glomerular tuft and renal blood vessels (red arrows) (Fig. E). The PG+ BB+GM group showing no histopathological alterations (Fig. F).

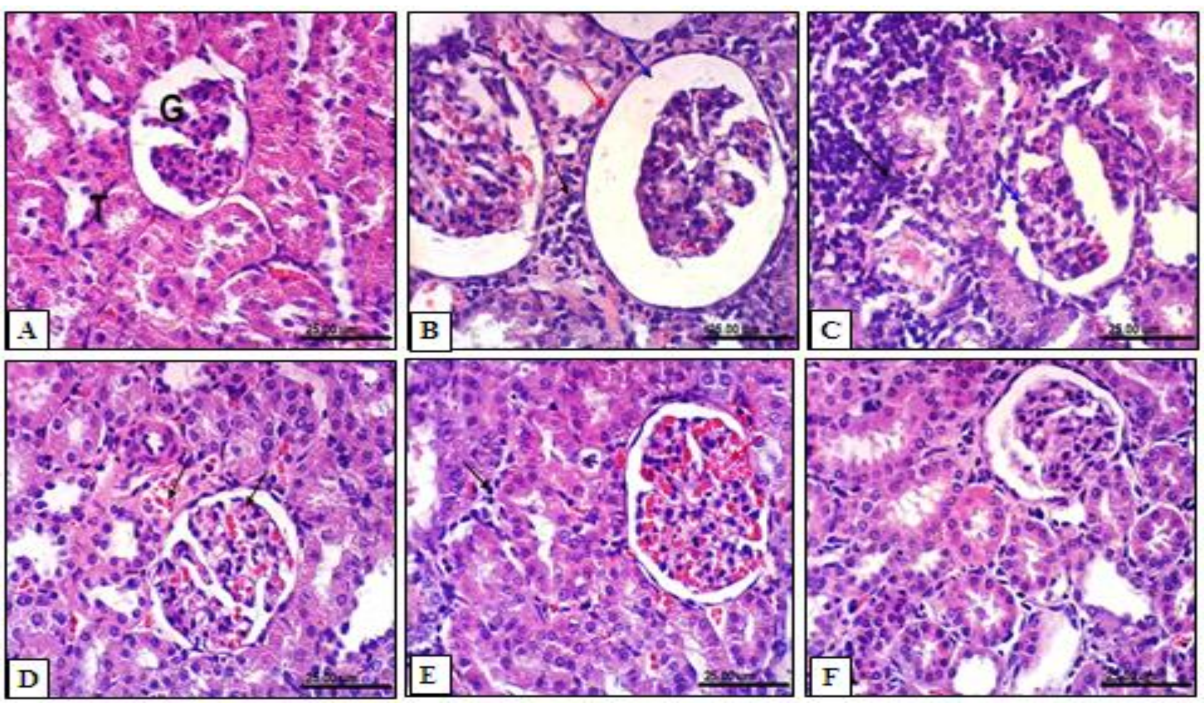

Figure 4 Impact of PG, BB and their combination on kidney histopathological alteration in nephrotoxic rats (H&E X400). A higher magnification of kidney sections of rats from the control group show normal histological structure of renal parenchyma, glomeruli (G) and renal tubules (T) (Fig. A). The GM group showing periglomerular inflammatory cells infiltration (black arrow), thickening of the parietal layer of Bowman's capsule (red arrow) and dilatation of Bowman's space (blue arrow) (Fig. B), besides marked interstitial nephritis with mononuclear inflammatory cells infiltration (black arrow) and hypercellularity of glomerular tuft (blue arrow) (Fig. C). PG+GM group show slight congestion of glomerular tuft and interstitial blood vessel (black arrows) (Fig. D). The BB+GM group show few inflammatory cells infiltration (black arrow) and congestion of glomerular tuft (red arrow) (Fig. E). The PG + BB + GM group showing no histopathological alterations (Fig. F).

4. DISCUSSION

The incidence of nephrotoxicity raised among populations around the world (Jain et al., 2013). The epidemiology of nephrotoxicity demonstrated that drug-induced nephrotoxicity accounts for 14-26% of kidney injury patients (Taber and Pasko, 2008). Therapeutic doses of aminoglycoside antibiotics as GM when given in long course (8 to >10 days) therapy makes the risk for nephrotoxicity higher by 20-25% in humans (Awodele et al., 2014). Therefore, GM has restricted of its clinical use these days because of its nephrotoxic effects.

Gentamicin injection induced statistical significant loss of BW in GM group, with decrease in FI relative to control group. This finding was agreed with Ezejiofor et al. (2014) and Khattab et al. (2016). The reduction in BW could be attributed to GM resulted in acidosis associated with anorexia that induced kidney damage, which lead to a lower BW as reported by (Houghton et al., 1976). Moreover, the increase catabolism accompanied by anorexia and the decrease of FI may be the causes of BW loss seen in acute renal failure induced by GM injection (Ali et al., 1992). On the other hand, BB and mixture PG+BB extracts significantly induced marked amelioration on BW as comparing with GM group. This finding was in disagreement with Wu et al. (2013) and Nair et al. (2014) who reported that, BB intake significantly decreased BW in obese rats fed with high-fat diet. Elks et al. (2011) reported that there were no significant differences of BWG in hypertensive rats. This variation of the results may be due to the different experimental protocol of rats in these previous studies, which were not nephrotoxic model. Our finding concerning the effect of BB against toxic effect of GM injection could be explained by the antioxidant protection effect of BB relate to its high flavonoids content, which act as growth promoter in rats by alternating nutrients absorption and metabolic utilization (El-Saeed et al., 2012). In addition, the increase in BW of nephrotoxic rats might be effected by the significant increase in FI of rats, which seen in this study.

Significant elevate in kidney weight in GM group relative to the control group was found. This finding was similar to the results of the previous studies reported by Ezejiofor et al. (2014) and Khattab et al. (2016). These results might be due to tissues damage, function alteration and edema caused by GM drug induced tubular necrosis (Rana et al., 2014). Pre-treatment with PG, BB extracts and their mixture in nephrotoxic rats resulted in significant decreased of kidney weight and relative weight of kidney relative to the GM group. These results agreed with the previous studies of Elks et al. (2011) and Mestry et al. (2020). Our results confirming by Shimeda et al. (2005) who stated that, the antioxidant silibinin and capsaicin produced a protective against cisplatin-induced nephrotoxicity and decreased kidney to body weight ratio.

The GM- nephrotoxic rats exert significant elevate in kidney function relative to the control group. The present findings agreed with the study of Sandhu et al. (2007); Ali and Saeed (2012); Boroushaki et al. (2014); Ezejiofor et al. (2014); and Mestry et al. (2020). These findings explained through toxic effect of GM that may influence the various metabolic kidney pathways. Gentamicin associated with oxidative stress and kidney injury, which causes various tubular dysfunction and electrolyte abnormalities ultimately lead to renal failure (Houghton et al., 1988). However, pre-treated rats showed significant decreased in all tested kidney function parameters relative to the GM group. The present findings were in agreement with those of Ali and Saeed (2012); Cekmen et al. (2013); Pan et al. (2019) and Mestry et al. (2020). The previous authors supported the effect of antioxidants in scavenging reactive oxygen metabolites and this property may contribute in enhancing renal functions via suppressing oxidative stress, which induced renal failure. Furthermore, Al-Sayed et al. (2015) revealed that the reduction of serum uric acid, urea, and creatinine levels may be related to the presence of many different phenolic compounds, which attributed to higher the potent antioxidant activity. The present results confirming that combination of PG and BB extracts in nephrotoxic rats showed the highest significant amelioration in all tested kidney function parameters.

Significant elevate in total protein and albumin in nephrotoxic rats relative to the control group was found. These findings is disagreement with Khan et al. (2011); Udupa and Prakash (2019); Mestry et al. (2020). However, there is an agreement with Khattab et al. (2016) results regarding albumin level. The increasing in albumin level may be explained by Khasanah et al. (2015) who concluded that GM induced disturbance in protein digestion or absorption. In addition, dehydration, where GM treatment for 10 days (100 mg/kg) induced significant higher quantity of urine volume, thus increases the urine volume which may cause dehydration (Khan et al., 2011; Khattab et al. 2016). The increasing in TP may be explained that TP plasma level contains albumin, globulin and fibrinogen. TP plasma level could be effected by albumin level since it has more than half of the composition in the plasma (Khasanah et al., 2015). The present study showed that administration of PG, BB, and their mixture extracts to GM injected rats significantly amelioration in serum level of TP and albumin as compared to GM group. These findings correlated with those obtained by Husain et al. (2018) and Mestry et al. (2020) who reported that consumption of methanolic extract of pomegranate leaves or juice, respectively by Wistar rats significantly ameliorated serum albumin levels.

There were decrease in Na^+ and K^+ after GM injection relative to the control group. This result is well documented by Zahid et al. (2013). Lower sodium level indicates kidney inability to conserve sodium (Padmini and Kumar, 2012). While lower potassium level may due to GM causing depression of apical membrane transporter, loss of brush border membrane enzymes and phospholipids

(Kaloyanides, 1984). A significant improvement was observed in all pre-treated groups as compared with the GM group in the serum level of Na^+ and K^+, the most effective pre-treatment was the mixture of PG+BB. These results were in accordance with Ali and Saeed (2012) that previously reported that the administration of PG extract at a dose level of (100 mg/kg) in male rats injected of GM (100 mg/kg) for 8 days showed significant improvement in plasma Na^+ and K^+ concentrations as compared to the GM group. The antioxidant activity of PG and BB could be attributed to its phenolic components, which may be responsible for decreasing oxidative stress and improvements kidney functions (Elks et al., 2011; Cekmen et al., 2013; Al-Sayed et al., 2015).

The biochemical results of this study were confirmed by histopathological findings. The kidney sections of the control group showed normal structure. While, the GM group showing severe damage alterations confined as marked interstitial nephritis with mononuclear inflammatory cells infiltration, cystic dilatation of renal tubules, hypercellularity of glomerular tuft, and dilatation of Bowman's space. The obtained results agreed with Mestry et al. (2020) who reported that GM caused severe inflammatory infiltrate in mononuclear cells. This may be due to the formation of highly reactive radicals as a consequence of oxidative stress caused by GM (Kumar et al., 2000). The histological findings of kidney tissue of the pre-treated rats showed slight regression of the histopathological lesions in PG+GM and BB+GM groups. Elks et al. (2011); Ali and Saeed (2012); Cekmen et al. (2013); Mestry et al. (2020) reported similar changes at different levels of dose of PG or BB. Furthermore, the mixture of PG+BB group showed the highest significant amelioration in all tested kidney sections; thus may be related to the presence of many different phenolic compounds, which attributed to higher the potent antioxidant activity (Al-Sayed et al., 2015).

5. CONCLUSION

Oral administration of PG, BB, and their mixture extracts at (500 mg/kg BW) have an ability to protect the kidney functions in nephrotoxic rats induced by GM. The mixture of BB+PG extract exerts the most nephroprotective effects than either BB or PG alone. Therefore, the combination of BB and PG extracts should be further investigated as a supplement to minimize or prevent the side effects of drugs-induced nephrotoxicity.

Ethical approval

This work was approved by the biomedical ethics research committee, Faculty of Medicine, KAU, ethical approval (Reference No (396-19). All procedures performed in the experimental study were in accordance with the ethical standards.

Funding

This study has not received any external funding.

Conflict of Interest

The authors declare that there are no conflicts of interests.

Data and materials availability:

All data associated with this study are present in the paper.

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